REMARKS

Claims 4-14 are pending.

Claims 1-3 and 15-18 have been withdrawn as being drawn to the non-elected Groups.

Applicants acknowledge the finality of the restriction requirement under 35 U.S.C. §121.

Reconsideration of the claim rejections and allowance of the application are requested consistent with the observations presented in the following.

In the Office Action of August 31, 2006, the Examiner rejected original claims 4-14 under 35 U.S.C. §103(a) based on a combination of prior art references submitted by Applicants. The Examiner found that it would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to use the immunoliposome assay as taught by Singh et al. (Anal. Chem., 2000) with nucleic acid reporters, as taught by Wu et al (Letters in Applied Microbiology, 2001) in order to detect the presence of an analyte in a sample with greater sensitivity than possible with standard immunoassay and fluorescence detection methods. Applicants disagree.

The Examiner contends that Singh et al teach a method for immunoliposome assay. Singh et al teaches a method for the incorporation of fluorescent phospholipid probes and ganglioside receptors into the bilayer of a liposome. Singh et al also teaches a method for using such modified liposomes as a detection reagent in a micrototer plate assay for certain biological toxins. (See page 6019, Col 1 line 22 and page 6024, Col. 2 line 30). Singh et al teaches toxin detection by way of the fluorescent signal produced by the liposome detection reagents, where its sensitivity corresponds to

that of conventional ELISA techniques (nanomolar/sub-nanomolar).

The Examiner acknowledges that Singh et al does not teach use of nucleic acid reporters/amplicons in the assay and that the assay does not describe, disclose, or suggest the use of PCR. Nevertheless, the Examiner proposes to combine Singh et al with Wu et al, a technical publication teaching use of nucleic acids and PCR in immuno-assays.

The Examiner's reliance on Wu et al in combination with Singh et al, in this case, is misplaced. The Examiner cited Wu et al as a basic immuno-PCR teaching for teaching use of nucleic acids as reporters for amplification, amplifying the nucleic acids, and detecting the nucleic acids as an indication of the presence of the analyte (see p. 322 col. 2 under Immuno-PCR assay lines 9-28 and p. 323 Figure 1). There is no dispute that Wu et al teaches a method for using the detection chimeras to detect antigens in a microplate assay for biological toxins. The method also teaches detection by way of PCR amplification of the reporters attached to the chimeras, that is, a method for producing a detection reagent by the covalent attachment of a short DNA segment (reporter) to an antibody. However, once so attached, *PCR is not possible*. Simply put, covalently linking "reporters" to antibodies would prevent amplification by PCR. Consequently, Wu et al does not teach reporters/amplicons being quantified PCR. Hence, without mutilating the teachings of Wu et al, it neither contemplates nor lends itself to immunoliposome-nucleic acid amplification assays of the type defined in the pending independent claims 4 and 12.

Turning to the patentability of the independent claims 4 and 12, first, commenting broadly, the invention combines aspects of divergent technologies drawn from membrane biophysics (liposomes), immunology (ELISA assay), and molecular biology (real-time PCR). Immuno-PCR researchers, like Wu et al, are aware of the problem of DNA contamination being a key limiting factor in assay sensitivity. Likewise, researchers in membrane biophysics, like Singh et al failed to recognize that liposomes could be used as a nucleic acid/amplicon vehicle in connection with immuno-PCR techniques. However, in the years/decades of co-existence of these disparate technologies, those of skill in the art in the immuno-PCR field failed to recognize that encapsulation of the reporters inside liposomes would be a way to abrogate the selectivity contamination problem, and those in the membrane biophysics field failed to recognize the use of amplifiable nucleic acid markers encapsulated with a liposome bilayer would significantly enhance assay sensitivity.

The present independent claims 4 and 12 define elegant and sophisticated inventions of fundamental distinction. First, both of the teachings of Singh et al and Wu et al, even if they could be combined as proposed by the Examiner, differ significantly in both steps and results. Simply put, there is no consideration or suggestion to rely on encapsulated nucleic acid markers. Secondly, the encapsulated markers, when freed from the liposomal bilayer are markers would not be capable of amplified as is required in the present invention. Thirdly, Singh et al teaches away from the encapsulation of the fluorocein markers by describing the leakage problem associated with such encapsulation. Fourthly, Wu et al teaches away from the use of free nucleic acids limiting the DNA to less than 40% in the conjugate. Finally, due to the reliance on amplification, not only does the

invention recited in claim 4 provide excellent selectivity but also provides remarkable sensitivity to as few as 10 molecules (sub-attomolar verses nanomolar) magnitudes beyond the Singh et al and beyond (sub-attomolar versus sub-femtomolar) Wu et al processes (see paragraph 30).

The present inventions of claims 4 and 12 permit detection and identification of proteins/toxins down to the 10-100 molecule level (See Paragraph 16, 28) while minimizing the repetitive amplification steps and minimizing undesirable effects of DNA contamination (See Paragraph 36). Another aspect of the inventions recited in claims 4 and 12 that is of practical significance arises from the fact of encapsulation of the nucleic acids. Because the markers are shielded from the encapsulation in the liposomes, DNAase/RNAase may be employed prior to amplification to minimize potential assay contamination from external sources (See Paragraph 17, 29). Thus, the inventions define methods capable of assay quantification of DNA reporters via the use of real-time PCR (See Paragraphs 28 and 59 of the application). Notably, such a practice in Wu et al would destroy the assay even if conjoined with the gangloiside projecting from the Singh et al bi-layer surface.

Applicants respectfully submit that, absent the disclosure of the instant invention, there is no suggestion or motivation in Singh et al or Wu et al to make the changes proposed by the Examiner. In other words, it appears that the Examiner engaged in hindsight reconstruction, relying on Appellant's disclosure, to selectively choose and alter bits and pieces from Singh et al with Wu et al.

> In order to establish a *prima facie* case of unpatentability, it is necessary for the examiner to present *evidence*, preferably in the form of some teaching, suggestion incentive or inference in the applied prior art, or in

> the form of generally available knowledge, that one having ordinary skill in the art *would have been led* to combine the teachings of the applied references in the proposed manner to arrive at the claimed invention [citations omitted]. *Ex parte Levengood*, 28 USPQ2d 1300, 1301 (BPAI 1993). (Emphasis in original)

Yet the Examiner suggests modifying the liposome assay of Singh et al with the PCR assay of Wu et al but without evidence supporting the assertion that Wu et al's nucleic acids could be encapsulated and retained without leakage into the Singh et al liposomes. Applicants submit that the Examiner's contention on this point is speculation. In *In re Jones*, 21 USPQ2d 1941, 1944 (Fed.Cir. 1992), the Federal Circuit reversed a rejection under 35 U.S.C. 103 noting that:

> "Conspicuously missing from this record is any evidence, other than the PTO's speculation (if it be called evidence) that one of ordinary skill in the ... art would have been motivated to make the modifications of the prior art ... necessary to arrive at the claimed [invention]." [Emphasis in original]

As acknowledged by the Examiner, Wu et al conjugates the reporter DNA-antibodies. Wu et al underscore the importance of covalent bonding of the antigen with the reporter DNA as a critical element of the described Immuno-PCR method (See page 324 Col 2) which contrasts to the Examiner's proposed substitution of the reporter DNA-antibody conjugates into the Singh et al liposomes. Wu et al do not teach the covalent attachment of antibodies to liposomes but only conjugation of the DNA to an antibody. Moreover, in the instant case, there is no evidence that the Singh et al method could be transformed into the claimed liposome PCR immunoassay. Because neither Singh et al nor Wu et al expressly or impliedly suggest the claimed invention, the Examiner must present a convincing line of

reasoning as to why the skilled artisan would have found the claimed invention to have been obvious in light of its teachings.

The only justification offered by the Examiner for combining the divergent references is found at page 6 of the Office Action:

> Wu et al. state, "...the method described here demonstrates that immuno-PCR technology greatly extends the sensitivity of immunoassays. This hybrid technology exhibited analyte detection from 100 to 1000 fold better than the ELISA method performed with the same antibodies. Immuno-PCR technology, in principle, provides the basis for a new generation of sensitive immunoassays and may be useful in clinicopathological assays as well as detection of low level antigens (see p. 325 col. 1 first full paragraph)," An ordinary practitioner would have been motivated to substitute the markers in the immunoliposome assay as taught by Singh et al. with nucleic acid reporters, as taught by Wu et al. in order to improve the sensitivity of the immunoliposome assay. The DNA reporters disclosed by Wu enable detection of analytes present in a sample at very low levels because the DNA markers improve sensitivity from 100 fold to 1000 fold over standard immunoassay methods, therefore the ordinary practitioner would expect a markedly higher degree of sensitivity in the immunoassay if the traditional fluorescence markers were substituted with the DNA reporters.

But this recitation does not appear to provide a *factual basis* for combining the references which, themselves, contain specific teachings that militate against such combination. The absence of a factual basis for making the proposed modifications adopted by the Examiner based on the prior art references, defeats an assertion of obviousness.

> It is incumbent upon the Examiner to provide a reason *why* one of ordinary skill in the art would have been led to modify a prior art reference or to combine the reference teachings to arrive at the claimed invention. *Ex parte Nesbit*, 25 USPQ2d 1817, 1819 (BPAI 1992)

Notably, the Examiner has associated improved sensitivity with that provide by ELISA techniques that may achieve sub-nanomolar detection, not sub-attomolar detection. Furthermore, in the recitation, the Examiner focused on the sensitivity but has not opined on the selectivity provided by the instant invention.

Notably, Wu et al admit to sensitivity problems when more than about 40% of the reporter DNA is cross-linked to form the conjugate presumably attributable to a steric hindrance problem "caused by the attachment of the relatively large DNA molecules near the antigen binding site". Wu et al describes a loss of sensitivity resulting from the use of too high a concentration of DNA- antibody conjugates (See page 323). In view of such teachings, Wu et al does not lend itself to, describe, contemplate or suggest nucleic acid markers encapsulated by a liposomal bilayer as an alternative to the described reporter DNA-antibody conjugates.

Moving to another issue relating to the Examiner's rejection, absent impermissible hindsight reconstruction, the invention of claims 4 and 12 remain patentable over the combination of Singh et al and Wu et al.

Reliance on Applicant's claims as a template for combining the cited references amounts to no more than impermissible hindsight. If unpatentability is predicated on hindsight reconstruction of the invention, the rejection cannot stand.

> At its core, our anti-hindsight jurisprudence is a test that rests on the unremarkable premise that legal determinations of obviousness, as with such determinations generally, should be based on evidence rather than on mere speculation or conjecture. Our court's analysis in Kahn bears repeating:
>
> A suggestion, teaching, or motivation to combine the relevant prior art teachings does not have to be found explicitly in the prior art, as "the teaching, motivation, or suggestion may be implicit from the prior art as a whole, rather than expressly stated in the references. . . . The test for an implicit showing is what the combined teachings, knowledge of one of ordinary skill in the art, and the nature of the problem to be solved as a whole would have suggested to those of ordinary skill in the art." However, rejections on obviousness grounds cannot be sustained by mere conclusory statements; instead, there must be some articulated reasoning with some rational underpinning to support the legal conclusion of obviousness. This requirement is as much rooted in the Administrative

> Procedure Act [for our review of Board determinations], which ensures due process and non-arbitrary decisionmaking, as it is in § 103.

Alza Corp. v. Mylan Labs., Inc., 464 F.3d 1286, 1289 (Fed. Cir. 2006), *citing In re Kahn*, 441 F.3d 977, 987 (Fed. Cir. 2006) (*quoting In re Kotzab*, 217 F.3d 1365, 1370 (Fed. Cir. 2000)).

> In order to establish a *prima facie* case of unpatentability, it is necessary for the examiner to present *evidence*, preferably in the form of some teaching, suggestion incentive or inference in the applied prior art, or in the form of generally available knowledge, that one having ordinary skill in the art *would have been led* to combine the teachings of the applied references in the proposed manner to arrive at the claimed invention [citations omitted]. *Ex parte Levengood*, 28 USPQ2d 1300, 1301 (BPAI 1993). (Emphasis in original)

In view of the foregoing, Applicant respectfully submits that the present invention as set forth in independent claims 4 and 12 are patentable over the combination of Singh et al and Wu et al (as well as over the remaining prior art of record).

The presently claimed inventions of claims 4 and 12, at once, increase sensitivity, dynamic range, and selectivity/accuracy of an assay relative to end-point PCR and detection of the amplified reporters by, for example, gel electrophoresis.

Summarizing Applicants' observations regarding the cited references, Wu et al does not teach the invention of claims 4 and 12 insofar as it does not disclose, discuss or suggest encapsulation of nucleic acid markers in liposomes. Singh et al uses liposomes but does not teach a method of nucleic acid encapsulation and actually teaches away from the concept of encapsulation of markers disassociated wit the liposomal bilayers.

Turning briefly, to secondary considerations supporting patentability, in this case the technical import of Applicants' invention has not gone without notice. The method has not only been subject to numerous publications in the technical press but even has been written up in the popular press. See Exhibits 1-6 attached hereto:

Science, Vol 309 (September 16, 2005)

Nature Protocols Vol 1. No. 4. 2003 (2006)

Nature Biotechnology, Advance Online Publication (2006)

Scientific American.com (April 17, 2006)

Chemical & Engineering News (May 1, 2006)

New York Times "Observatory" (April 25, 2006)

Such widespread recognition focusing on the technical merit of the invention, clearly supports patentability of the claims 4 and 12. That is, the publications emphasize the selectivity and sensitivity capabilities of the Applicants' inventive assay which is defined by claims 4 and 12. Indeed, in view of peer reviewed publications directed to the technical merit of the invention, Applicants' traverse the Examiner's finding on page 6 that

> It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to use the immunoliposome assay as taught by Singh et al. with nucleic acid reporters, as taught by Wu et al. in order to detect the presence of an analyte in a sample with greater sensitivity than possible with standard immunoassay and fluorescence detection methods.

In the face of the noted widespread recognition, the combination of Singh et al with Wu et al to defeat the patentability of the claimed invention is belied.

Claims 5-11 and 13-14 likewise, remain patentable over the prior art of record at a minimum due to their dependence respectively on independent claims 4 and 12.

Based on the foregoing, Applicants submit that claims 4 and 12 are patentable over the prior art of record. Where an independent claim is free of the art, then any claim depending therefrom is also free of the art. *In re Fine,* 837 F.2d 1071, 1075 (Fed. Cir.1988). Accordingly, Applicants submit that the inventions recited in dependant claims 5-11 and 13-14 are likewise allowable over the art of record.

In view of the remarks above, Applicants respectfully submit that the present application is in condition for immediate allowance with at least claims 4-14 and such action is hereby solicited.

If in the opinion of the Examiner, a telephone conference would expedite the prosecution of the subject application, the Examiner is invited to call the undersigned.

Respectfully submitted,
CAHN & SAMUELS, LLP

January 2, 2007

/muc/
Maurice U. Cahn, Reg. No. 30, 454
2000 P Street, N.W. (Suite 200)
Washington, D.C. 20036
Telephone: (202) 331-8777